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Formulation And Development Of Metronidazole Loaded Niosomal Formulation For Sustained Delivery

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Abstract

Objective: The main objective of this study is to design suitable metronidazole loaded niosomes for rapid onset of action with lesser side effects and also to overcome the insufficient localization of a drug to the liver.
Methods: Metronidazole niosomes are prepared by using Thin film hydration technique. Span60 is used as non-ionic surfactant and cholesterol is used as stabilising agent in this method. Size of niosomes is estimated by using optical microscopy. Percentage drug entrapment efficiency is calibrated by using UV-Spectrophotometer. The in-vitro release studies for the drug Metronidazole were conducted by using dialysis method.

Results: The parameters studied in this research are percentage drug release for entrapment efficiency and In-vitro release studies. Stability studies, SEM (Scanning electron microscopy) analysis, IR studies.

Conclusion: The results conclude that the metronidazole niosomal formulation produce optimal therapeutic effect with lesser side effects.

Keywords: Metronidazole Niosomes, Entrapment efficiency, Thin film hydration technique, SEM, UV spectrophotometer, PBS (Phosphate buffer solution),

Introduction

Vesicular delivery systems are novel drug delivery systems and provides target specific and controlled release of drugs. There are different types of vesicular systems they are liposomes, niosomes, transferosomes, pharmacosomes and ethosomes [1]. Among these carriers Niosomes are best carriers of drugs because they are more stable, biodegradable, economical, non-toxic, biocompatiable, insucesptible, and show pliability according to their structural characteristics [2-5].

Niosomes are non-ionic surfactant vesicles foremostly announced in 1970's by the scholars of cosmetic industry [6-8]. Niosomes are obtained by a hydrating mixture of cholesterol and a non-ionic surfactant [9]. Niosomes can entrap both hydrophilic and lipophilic drugs and can prolong the circulation of the entrapped drug in body [10-11].

Regarding the structure of niosomes it is identical to that of liposomes which also consists of bilayer like that of liposomes but the difference is that noisome consists of non-ionic surface-active agents while liposomes have phospholipids [12]. The stability of niosomes are influenced by type of surfactant, nature of encapsulated drug, storage temperature, detergents, use of membrane spanning lipids, in order to keep niosomes stable the above parameters should be under control [13]. These niosomes can act as a depot and helps in sustain release of drug [14]. Niosomes are classified into different types based on number of bilayer (e.g. MLV- Multilamellar vesicles, SUV- Small unilamellar vesicles) or base on size (e.g. LUV- Large unilamellar vesicles, SUV) [15]. Different methods are used to prepare niosomes and they include the following reverse phase evaporation technique [16], film hydration technique [17], ether injection method [18-19], hand shaking method, lipid layer hydration method, trans membrane pH gradient method [20].

Metronidazole is a limited spectrum synthetic nitroimidazole derivative used in the treatment of many anaerobic, protozoan and parasitic infections [21][22]. This article is concentrating on treatment of hepatic amoebiasis by using metronidazole niosomes. Conventional delivery of metronidazole in a treatment of Hepatic Amoebiasis is limited and ineffective concentration is achieved in the liver [23]. Metronidazole conventional dosage forms may cause side effects like anorexia, nausea, coated tongue, and dizziness, malaise, fever, and abdominal pain [24]. It undergoes first pass metabolism and leads to decrease in bioavailability [25]. In the aspect of overcoming the adverse effects caused by conventional doses a novel drug delivery system like niosomes can be used.

Materials and Methods

Materials

Materials used in the formulation are Metronidazole (Max med laboratories), Span 60 (SRL SISCO; Chennai) Cholesterol (SRL SISCO; Chennai) Ether (SRL SISCO; Chennai) Chloroform (SRL SISCO; Chennai) Dialysis membrane (SRL SISCO; Chennai).

Equipment used in obtaining formulation are weighing balance (Shimadzu-AY220,JAPAN), dissolution test apparatus (Labindia disso 1800, Mumbai), UV/Visible spectrophotometer (Shimadzu-1700, Japan), probe sonicator (Rivotek, Mumbai), bath sonicator (Sicon, New Delhi), scanning electron microscope(Quanta FEG, USA) and centrifuge (Industrial and laboratory Chennai).

Preparation of standard curve for Metronidazole

100mg of Metronidazole drug was weighed accurately and transferred into a 100ml standard flask and 50ml of 0.1M HCl is added to solubilise the drug. Then the volume is made up to 100ml with 0.1M HCl. This gives the stock solution.

The above stock solution is diluted with 0.1M HCl to give the concentrations of 20, 40, 60, 80 and 100µg/ml. Then the absorbance of the solution is measured at 200-400nm by using UV-visible spectrometer and a graph is plotted by taking concentration on X-axis and absorbance on Y-axis to obtain a standard curve. The standard curve used to estimate the concentration of the drug metronidazole.

FTIR Spectral Analysis

Compatibility studies were conducted for Metronidazole and its excipients (cholesterol and non-ionic surfactants) by using FTIR spectroscopy in order to find out any the interaction present between them.

Preparation of Metronidazole Niosomes

In this preparation Metronidazole, non-ionic surfactant and cholesterol were weighed

accurately and dissolved in chloroform and methanol system (1:2) in a round bottom flask. The solvent mixture was evaporated in a rotary evaporator, under vacuum at 27 $\pm$ 2ºC at 100 rpm until a dry white lipid film was obtained. The obtained film was hydrated with 20ml phosphate buffer solution with pH 7.4 for an hour in round bottom flask at 100 rpm until a white suspension is obtained. The obtained niosomal suspension was hydrated at 4ºC in the absence of light for 24 hours. The technique used in the preparation of metronidazole niosomes is called as Thin film hydration technique. Formulations of Niosomes is given in Table 1 and stability of formulations is given in Table 2.

Evaluation of Niosomes

Drug content: Niosomal suspension equivalent to 10mg (0.01) taken in a standard volumetric flask of 100 ml and volume was made up to 100ml with phosphate buffer of pH 7.4 after that 1ml of this mixture was pipette out and diluted to 20ml by phosphate buffer pH 7.4 and the percentage drug content was observed at 230nm using UV spectrophotometer.

Percentage drug entrapment (PDE): The entrapped Metronidazole within Niosome was determined after removing the unentrapped drug by dialysis. The dialysis was carried out by taking niosomal dispersion in dialysis bag, which was dipped in a beaker containing 250ml of PBS with a pH of 7.4; the beaker was placed on a magnetic stirrer and run for 8 hrs with a speed of 80-120 rpm. Then the solution inside the receptor compartment was studied for unentrapped Metronidazole at 200-400 nm using Spectrophotometer. The PDE in the noisome was calculated from the ratio of the difference of the total amount of drug added and the amount of unentrapped drug detected, to the total amount of drug added.

$$\text{Percent entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100$$

In vitro Drug release: The release of metronidazole from niosomal formulations were studied using a dialysis method. Dialysis bags were soaked in PBS before use at room temperature for 12 hrs to remove the preservative, followed by rinsing thoroughly in distilled water. In vitro release of metronidazole from niosomes was conducted by dialysis in a dialysis sac made up of a cellophane membrane. The niosomal formulation equivalent to 100mg of metronidazole was placed in a dialysis membrane of diameter 2.5cm with an effective length of 8cm that acts as donor compartment. Two ends of the dialysis sac were tightly bound with threads. The sac was placed in a beaker containing 250 ml of Phosphate buffer (pH7.4) which acts as receptor compartment. The beaker was kept on a magnetic stirrer and stirring was maintained at 100rpm at 37 °c with thermostatic control. 5ml samples were collected at 1h interval over a period of 6h and assayed spectrophotometrically at 277 nm for drug released and the sampled volume of buffer maintained at the same temperature. An equal volume of fresh release medium was replaced at the same intervals. The diffusion data were analyzed for calculating the amount of drug released and percentage drug released at different time intervals.

Stability Studies: The niosomal formulations were kept in air tight containers and stored at refrigeration temperature (2-8˚c) and at room temperature (25±2˚c) for 30 days. After 30 days shape, entrapment efficiency and drug content were measured and the result was compared with the initial sample.

Scanning Electron Microscopy: Particle size of niosomes is a very important characteristic. By using scanning electron microscopy, the surface morphology (roundness, smoothness, and formation aggregates) and the size distribution of niosomes was studied. Niosomes were sprinkled on to the double sided tape that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of the scanning electron microscope. The samples

were observed for morphological characterization using a gaseous secondary electron detector.

Results and Discussion

Standard curve for Metronidazole: Figure 1 depicts the standard curve graph of metronidazole.

FTIR Spectral Analysis: Figures 2, 3, and 4 shows the IR spectra. By observing the spectra, we came to see that the peaks of metronidazole excipients are similar to that of the peaks of metronidazole niosomes. Thus, from the spectra it was understood that there was no interaction between Metronidazole and excipient used in the preparation of Metronidazole niosome.

Scanning Electron Microscopy: SEM Analysis is one of the important parameters which gives the morphology of Niosomes. SEM of Metronidazole niosome is shown in the Figure 5.

In-vitro drug release at 8 hours: In-vitro drug release studies are shown from Table 3 to Table 6. From the results, we observed that MET1 formulation gives highest percent drug release at the end of 8 hours. So the formulations having 1:1 ratio of cholesterol and span60 gives optimum formulation. Hence, drug: surfactant: cholesterol (100: 50: 50) MET1 formulation gives the optimum release. The same can be observed from Figure 6 of in-vitro drug release studies.

Entrapment efficiency: The drug entrapment efficiency is given in Table 7 which ranges from 72% to 85%. These entrapment efficiencies are compared with In-vitro drug release and drug content. The entrapment efficiency is shown in Figure 7 and its comparison with in-vitro release and drug content is shown in Figure 8 which shows that that formulation having highest drug entrapment efficiency is having the highest in-vitro drug and drug content which is MET1 formulation.

Conclusion

Niosomal formulations having the concentration of 100:50:50 is considered to be the stabilized formulation with a higher entrapment efficiency and better drug release. The higher metronidazole entrapment was observed with non-ionic surfactant/cholesterol at ratio 1:1. Thus, MET1 formulation is considered as a more reliable formulation that is needed to ensure effective quantities of metronidazole to be delivered in systemic circulation. The average particle size for niosomal formulations was found to be 400-500μm and were found to be spherical and discrete in nature. Hence, these metronidazole formulations will be better targeted and reduce the dose of metronidazole and decrease the frequency of administration and provide better therapeutic activity in lesser doses, thereby decreasing the side effect caused by the conventional drug delivery system.

References

1. Rajera, R., Nagpal, K., Singh, S. K., & Mishra, D. N. (2011). Niosomes: a controlled and novel drug delivery system. Biological and Pharmaceutical Bulletin, 34(7), 945-953.
2. Lohumi, A. (2012). A novel drug delivery system: niosomes review. Journal of drug delivery and therapeutics, 2(5).
3. Choi, M. J., & Maibach, H. I. (2005). Liposomes and niosomes as topical drug delivery systems. Skin pharmacology and physiology, 18(5), 209-219.
4. Shilakari Asthana, G., Sharma, P. K., & Asthana, A. (2016). In vitro and in vivo evaluation of niosomal formulation for controlled delivery of clarithromycin. Scientifica, 2016.
5. Kazi, K. M., Mandal, A. S., Biswas, N., Guha, A., Chatterjee, S., Behera, M., & Kuotsu, K. (2010). Niosome: a future of targeted drug delivery systems. Journal of

advanced pharmaceutical technology & research, 1(4), 374.
6. Uchegbu, I. F., & Vyas, S. P. (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. International journal of pharmaceutics, 172(1-2), 33-70.
7. Mahale, N. B., Thakkar, P. D., Mali, R. G., Walunj, D. R., & Chaudhari, S. R. (2012). Niosomes: novel sustained release nonionic stable vesicular systems—an overview. Advances in colloid and interface science, 183, 46-54.
8. Biswal, S., Murthy, P. N., Sahu, J., Sahoo, P., & Amir, F. (2008). Vesicles of non-ionic surfactants (niosomes) and drug delivery potential. International journal of pharmaceutical sciences and nanotechnology, 1(1), 1-8.
9. Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., & Rogerson, A. (1985). The preparation and properties of niosomes—non-ionic surfactant vesicles. Journal of pharmacy and pharmacology, 37(12), 863-868.
10. Jain, A. P., Sharma, P., Pandey, P., Gupta, R., Roshan, S., Garg, A., ... & Jain, A. (2013). Niosome a novel approach for drug delivery system: an overview. Asian J Pharm Sci Res, 3(5), 18-30.
11. Sankhyan, A., & Pawar, P. (2012). Recent trends in niosome as vesicular drug delivery system. Journal of Applied Pharmaceutical Science, 2(6), 20-32.
12. Makeshwar, K. B., & Wasankar, S. R. (2013). Niosome: a novel drug delivery system. Asian journal of pharmaceutical research, 3(1), 16-20.
13. Ceren Ertekin, Z., Sezgin Bayindir, Z., & Yuksel, N. (2015). Stability studies on piroxicam encapsulated niosomes. Current Drug Delivery, 12(2), 192-199.
14. Fang, J. Y., Hong, C. T., Chiu, W. T., & Wang, Y. Y. (2001). Effect of liposomes and niosomes on skin permeation of enoxacin. International journal of pharmaceutics, 219(1-2), 61-72.
15. Makeshwar, K. B., & Wasankar, S. R. (2013). Niosome: a novel drug delivery system. Asian journal of pharmaceutical research, 3(1), 16-20.
16. Guinedi, A. S., Mortada, N. D., Mansour, S., & Hathout, R. M. (2005). Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. International journal of pharmaceutics, 306(1-2), 71-82.
17. Bayindir, Z. S., & Yuksel, N. (2010). Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery. Journal of pharmaceutical sciences, 99(4), 2049-2060.
18. Aggarwal, D., Garg, A., & Kaur, I. P. (2004). Development of a topical niosomal preparation of acetazolamide: preparation and evaluation. Journal of Pharmacy and Pharmacology, 56(12), 1509-1517.
19. Jain, C. P., & Vyas, S. P. (1995). Preparation and characterization of niosomes containing rifampicin for lung targeting. Journal of microencapsulation, 12(4), 401-407.
20. Bhaskaran, S., & Lakshmi, P. K. (2009). Comparative evaluation of niosome formulations prepared by different techniques. Acta Pharmaceutica Sciencia, 51(1).
21. Stan, C. D., Tataringa, G., Gafitanu, C., Dragan, M., Braha, S., Popescu, M. C., ... & Stefanache, A. (2013). Preparation and characterization of niosomes containing metronidazole. Farmacia, 61(6), 1178-1185.
22. Tanowitz, H. B., Wittner, M., Rosenbaum, R. M., & Kress, Y. (1975). In vitro studies on the differential toxicity of metronidazole in protozoa and mammalian cells. Annals of Tropical Medicine & Parasitology, 69(1), 19-28.
23. Saravanan, D., & Popli, H. (1998). Preparation and Evaluation of Metronidazole-loaded Niosomes in Rats. Pharmacy and Pharmacology Communications, 4(10), 485-487.
24. Antani, J., & Srinivas, H. V. (1970). Clinical evaluation of metronidazole in

hepatic amebiasis. American Journal of Tropical Medicine and Hygiene, 19(5), 762-6.
25. Lau, A. H., Lam, N. P., Piscitelli, S. C., Wilkes, L., & Danziger, L. H. (1992). Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. Clinical pharmacokinetics, 23(5), 328-364.

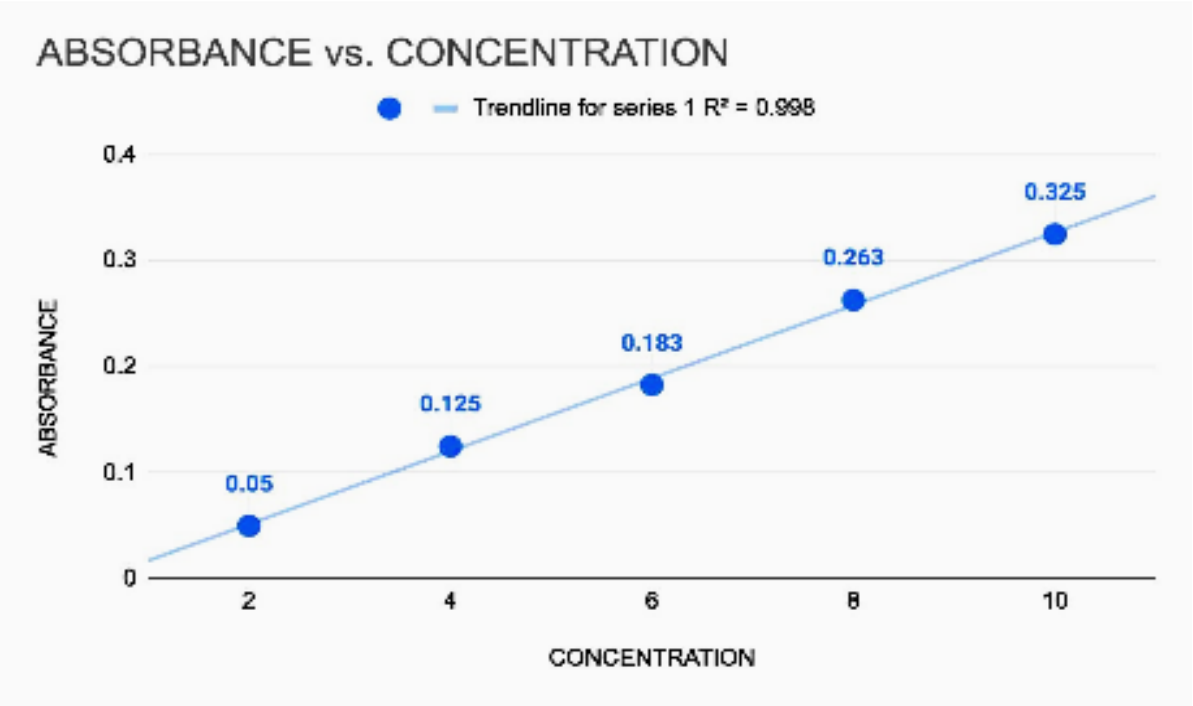
Table 1: Formulation of Niosomes

Formulation	Drug	Span 60	Cholesterol
MET1	100	50	50
MET2	100	50	25
MET3	100	100	50
MET4	100	100	25

Table 2: Stability analysis

DURATION	**FORMULATION**	**REFRIGERATED TEMPERATURE**
For 1 week	MET 1 MET2 MET3 MET4	Spherical shape (No clumping)
After a month	MET 1 MET2 MET3 MET4	Spherical shape Sedimentation and slight clumping

Figure 1: Standard curve of Metronidazole



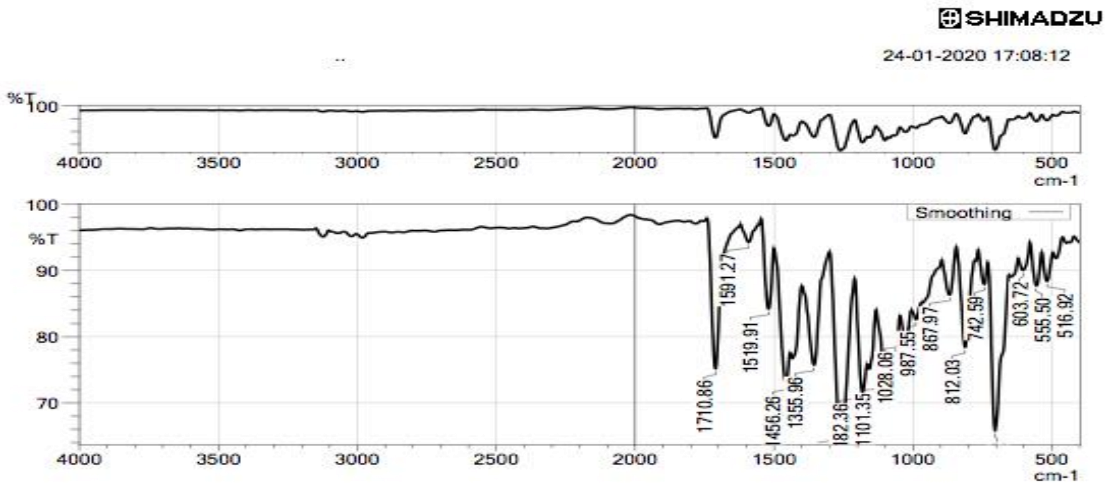


Figure 2: IR Spectra of Metronidazole

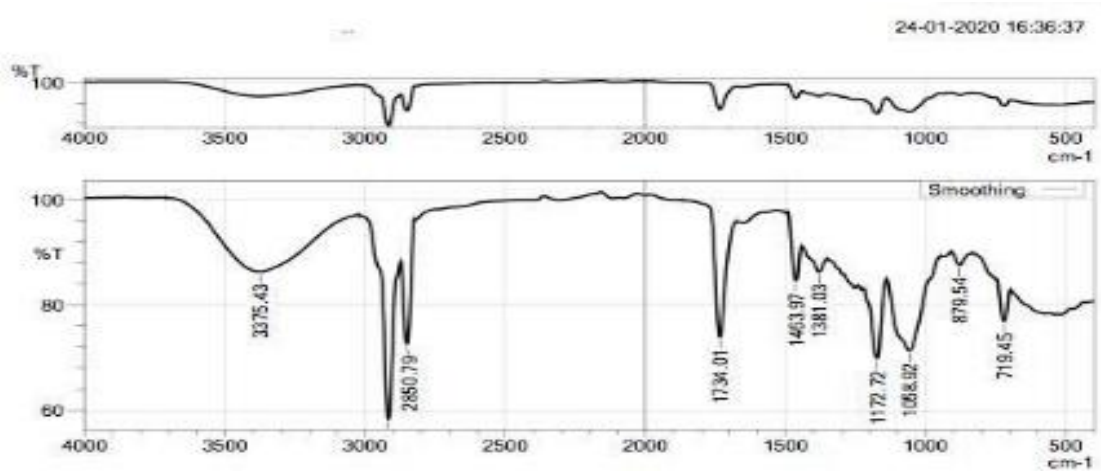


Figure 3: IR Spectra of Cholesterol

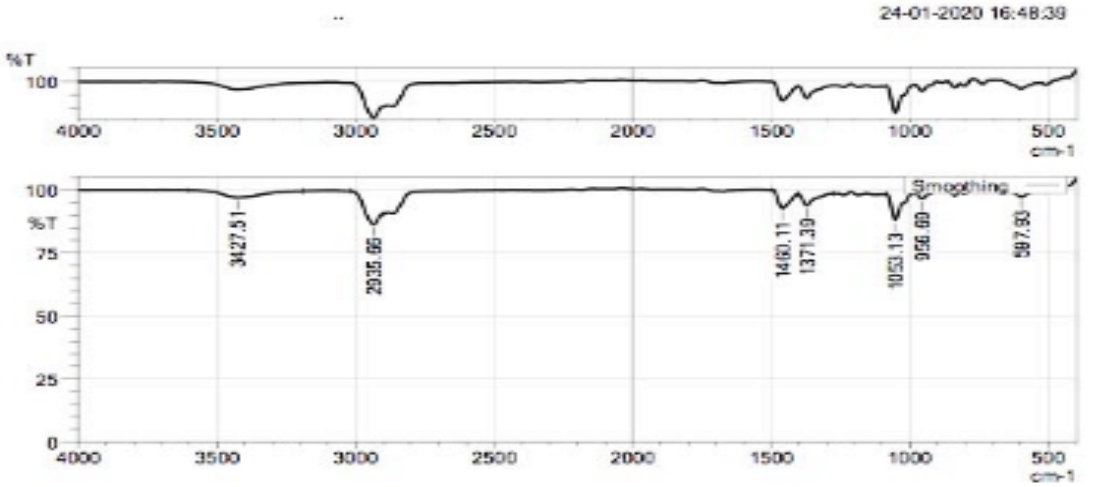


Figure 4: IR spectrum of Span60

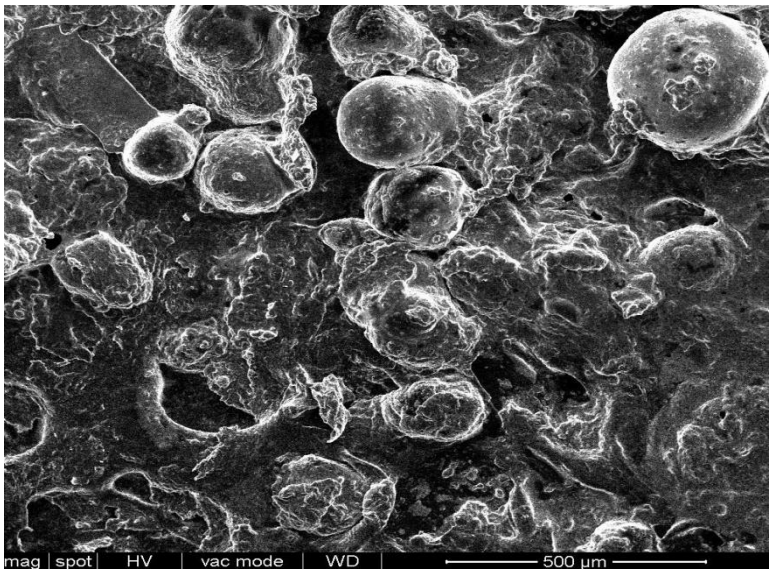


Figure 5: SEM of MET1

Table 3: In vitro release profile of Metronidazole for MET1

Time (hours)	Absorbance	In -Vitro concentration	Amount of drug release	% drug release
1	0.0326	0.24	0.323	32.3
2	0.0354	0.26	0.496	49.6
3	0.0387	0.28	0.534	53.4
4	0.0410	0.30	0.741	74.1
6	0.0438	0.32	0.872	87.2
8	0.0466	0.34	0.943	94.3

Table 4: In vitro release profile of Metronidazole for MET2

Time (hours)	Absorbance	In-vitro concentration	Amount of drug release	% drug release
1	0.0200	0.15	0.374	37.4
2	0.0214	0.16	0.404	40.4
3	0.0242	0.18	0.514	51.4
4	0.0256	0.19	0.738	73.8
6	0.0270	0.20	0.884	88.4
8	0.0284	0.21	0.901	90.1

Table 5: In vitro release profile of Metronidazole for MET3

Time (hours)	Absorbance	In-vitro concentration	Amount of drug release	% drug release
1	0.0144	0.11	0.153	15.3
2	0.0158	0.12	0.223	22.3
3	0.0200	0.15	0.366	36.6

4	0.0214	0.16	0.438	43.8
6	0.0242	0.18	0.543	54.3
8	0.270	0.20	0.884	88.4

Table 6: In vitro release profile of Metronidazole for MET4

Time (hours)	Absorbance	In- vitro concentration	Amount of drug release	%drug release
1	0.0163	0.124	0.384	38.4
2	0.0180	0.136	0.492	49.2
3	0.0200	0.156	0.574	57.4
4	0.0230	0.171	0.774	77.4
6	0.0250	0.188	0.893	80.3
8	0.0270	0.200	0.806	89.6

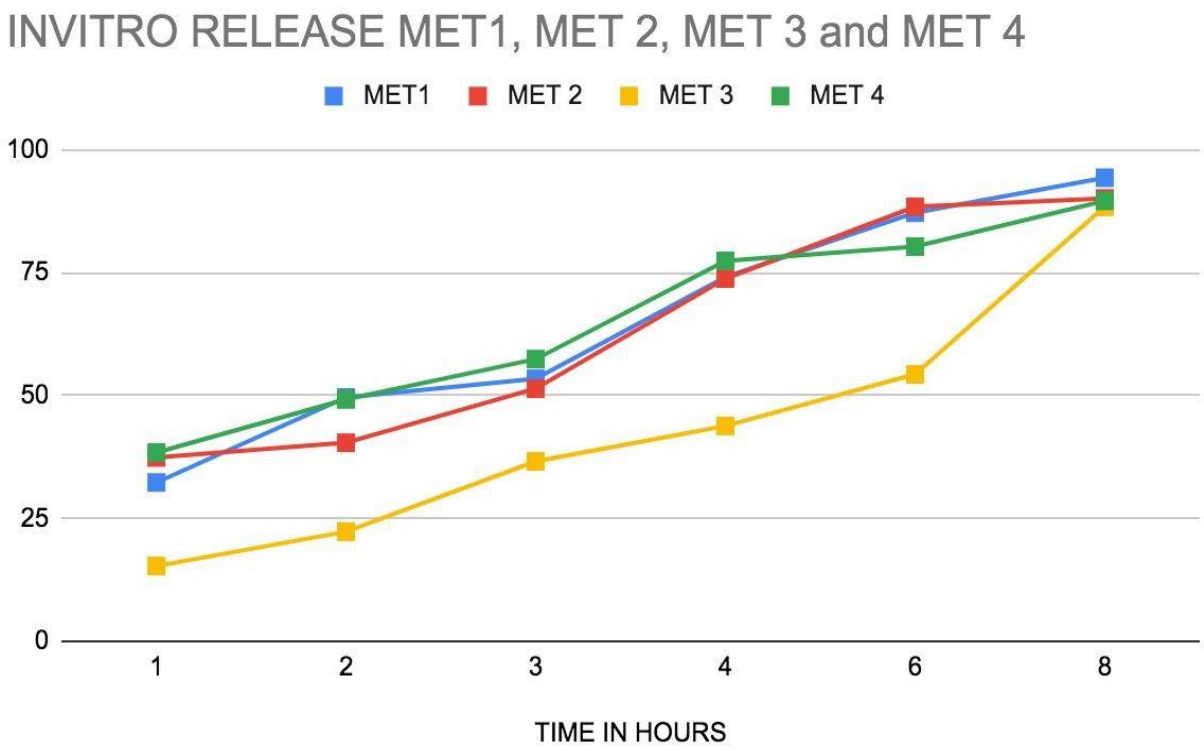


Figure 6: In vitro release profile of Metronidazole for different batches of drug loaded

Table 7: Entrapment efficiencies

Code	% Entrapment efficiency	In-Vitro Release	Drug Content
MET1	85	94.3	92
MET2	80	90.1	88
MET3	72	87.9	78
MET4	76	89.3	84

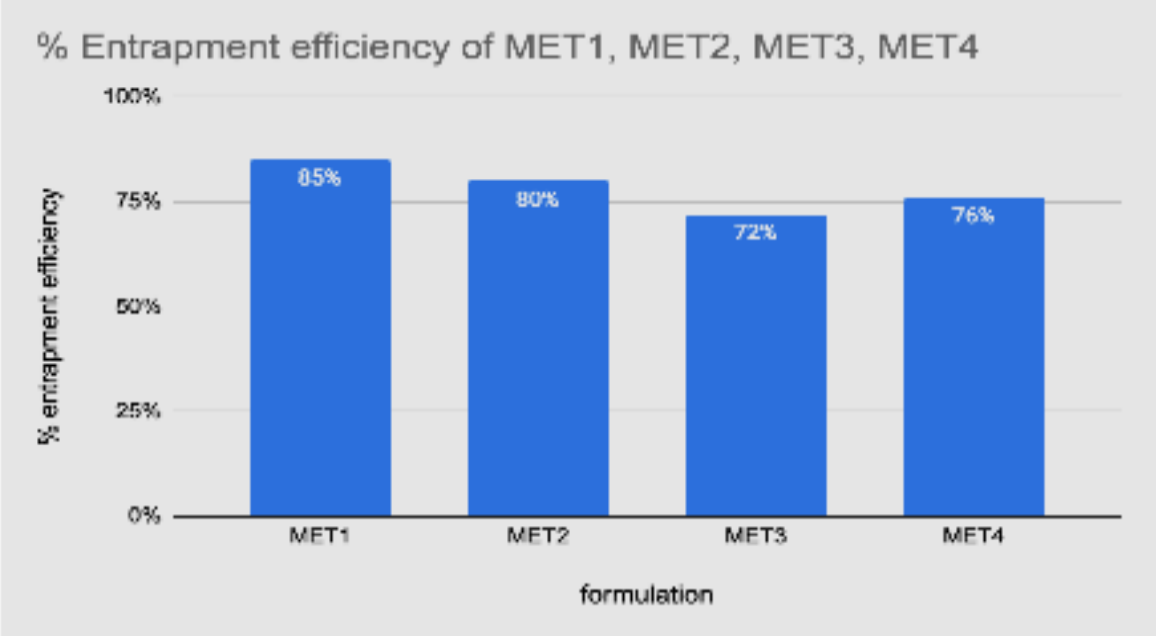


Figure 7: % Entrapment efficiency of MET 1, MET2, MET 3, MET 4

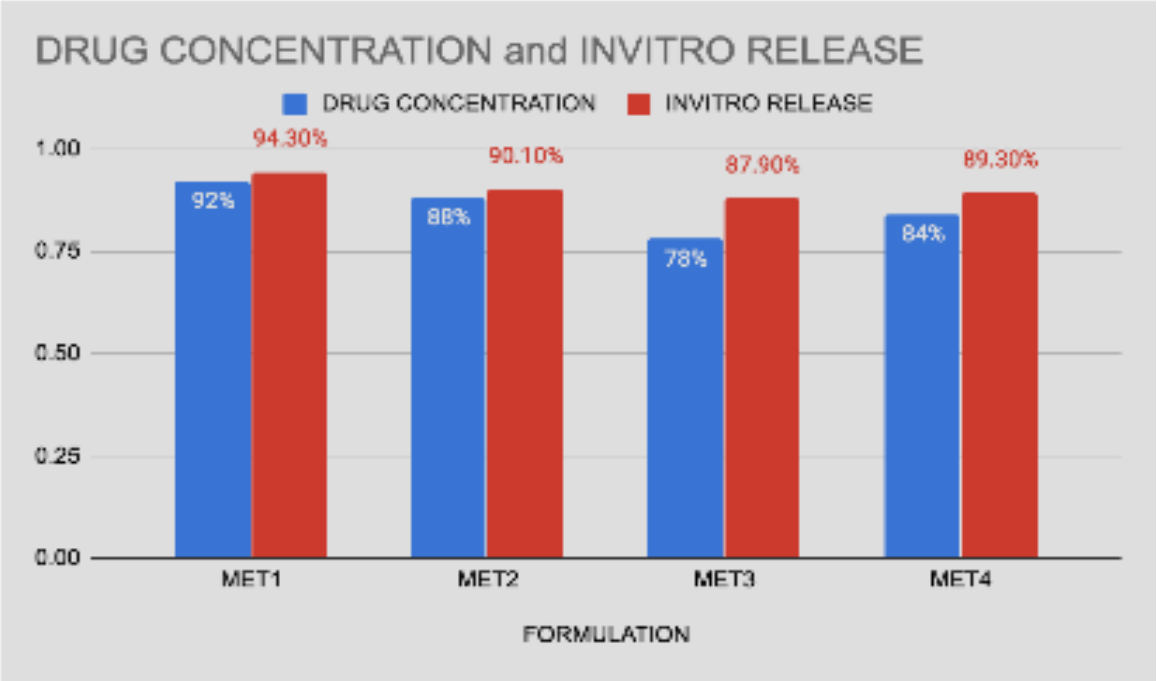


Figure 8: Drug Concentration And In Vitro Release